Synthesis, Crystal Structure and Antitumor Activities of Ethyl (R)-2-(Biphenyl-4-carbonyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-1-carboxylate

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ABSTRACT The title compound, ethyl (R)-2-(biphenyl-4-carbonyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-1-carboxylate ($C_{27}H_{24}N_2O_3$) has been synthesized, and its structure was characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-MS and single-crystal X-ray diffraction. It crystallizes in the orthorhombic system, space group *Pbca* with a = 16.9950(8), b = 9.5445(4), c = 28.3188(3) Å, V = 4593.6(3) Å^3, Z = 8, T = 294.64(10) K, μ(Mo$K\alpha$) = 0.08 mm^{-1}, D_c = 1.228 g/cm^3, F(000) = 1792.0 and *GOOF* = 1.036. 11836 reflections were measured (7.04≤2θ≤52.04°), and 4506 were unique (R_{int} = 0.0393, R_{sigma} = 0.0546) and used in all calculations. The final R = 0.0576 ($I > 2\sigma(I)$) and wR = 0.1563 (all data). The preliminary biological tests show that the title compound has a good antitumor activity against Hela *in vitro* with the IC_{50} value of 4.71 μmol/L.

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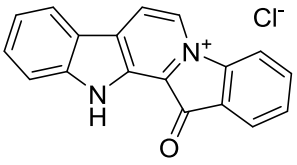
1 INTRODUCTION

Fascaplysin (Scheme 1), a natural product originally isolated from a marinesponge, *Fascaplysinopsis Berquistsp*[1], is known to possess antimicrobial, antimalarial, and antiacetylcholinsteres activities[2-4]. Moreover, it is reported that fascaplysin also can be shown to block the growth of cancer cells presumably through the inhibition of cyclin-dependent kinase 4(CDK4), an early cell cycle enzyme misregulated in most cancers[5]. However, fascaplysin shows high toxicity at the cellular level, which could be attributed to its planar structure to bind and intercalate into the structure of DNA[6].



Fascaplysin

Scheme 1. Structure of fascaplysin

In view of these facts mentioned above, many efforts have been devoted to the synthesis of a series of non-planar fascaplysin-based derivatives[7-11]. Our laboratory is also doing this work. The main goal of our current study is to develop potent, non-planar CDK4 specific analogues of fascaplysin, and they do not intercalate or interact with the minor groove of double-stranded DNA. Our strategy is to open the pyrrole ring of fascaplysin and retain the scaffold of *β*-carboline to design low toxic and high efficient derivatives, and the title compound was designed as a novel derivative of fascaplysin showing good antitumor activities. In this paper we describe the synthesis of this compound by using tryptamine as the starting material (Scheme 2), and also focus on its crystal structure and antitumor activity.

2 EXPERIMENTAL

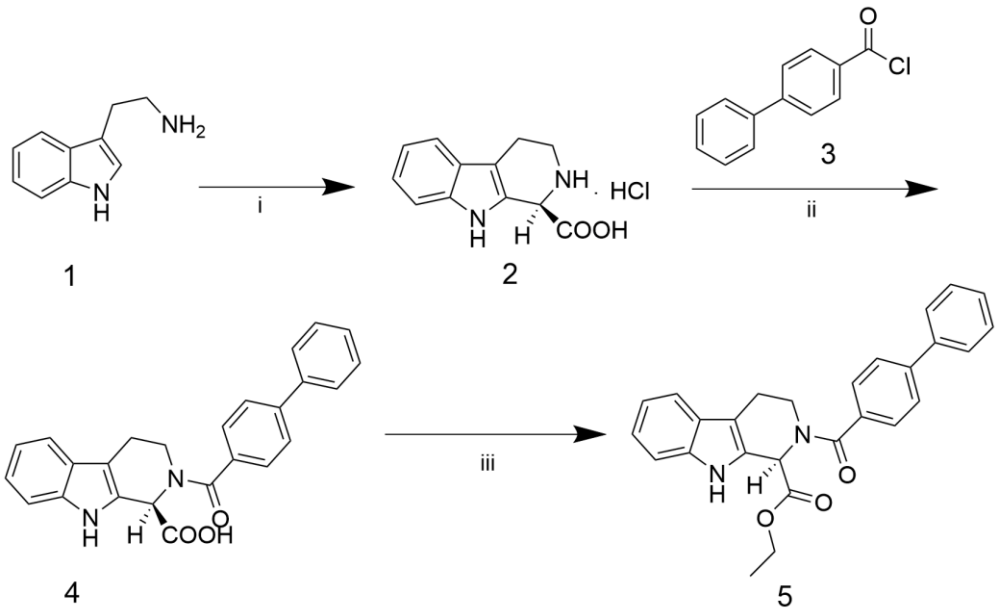
2. 1 Instruments and reagents

All organic solvents and materials were obtained from commercial suppliers and used with further purification. Melting points were determined by using the electrothermal PIF YRT-3 apparatus without correction. ^{1}H-NMR and ^{13}C-NMR (δ ppm) spectra were measured on a Varian Mercury (400MHz) using TMS as the internal standard. Mass spectra were recorded on a VGZAB-HS (70 ev) spectrometer with ESI source as ionization. Crystallographic data of this compound were collected by a Super Nova, Dual, Cu at zero, Eos diffractometer.

2. 2 General procedure

The tryptamine (**1**, 1.46 g, 10 mmol) was chosen as the starting material for this experiment, which was converted into compound **2** (white solid, 90% yield) according to the Pictet-Spengler reaction in good yield[12, 13]. And compound **3** (0.520 g, 2.4 mmol) dissolved in 5 mL dry 1,4-dioxane and a 2 N aqueous sodium hydroxide (2 mL) were added to a solution of **2** (0.51 g, 2 mmol) in a mixed solvent of 2 N aqueous sodium hydroxide (2 mL) and 1,4-dioxane (2 mL), followed by stirring at 0 °C for 20 minutes. The reaction mixture

was stirred at room temperature for 20 hours, then acidified with concentrated hydrochloric acid to obtain a solid product, which was washed with water and dried to obtain compound **4** (light yellow solid, 85% yield). After that, thionylchloride (0.24 g, 2 mmol) was slowly dropwise poured into the solution of **4** in 6 mL anhydrous ethanol and stirred for 30 minutes in an ice bath. Then the mixture was stirred for 8 h at room temperature and monitored by TLC. After filtration, the filtrate was concentrated under reduced pressure, and the residue was purified via silica gel column chromatography (ethyl acetate/petroleum ether = 1/3). Then compound **5** (white solid, 72% yield) was obtained, m.p.: 201.4～201.8 ℃. ^{1}H-NMR (400 MHz, DMSO-d_6, TMS, ppm): δ 11.05 (s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 7.3 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.52 (t, J = 7.6 Hz, 2H), 7.43 (dt, J = 7.3, 6.9 Hz, 3H), 7.12 (t, J = 7.7 Hz, 1H), 7.01 (t, J = 7.4 Hz, 1H), 6.05 (s, 1H), 4.30～4.20 (m, 2H), 4.03 (dd, J = 13.9, 3.9 Hz, 1H), 3.59 (dd, J = 17.8, 7.8 Hz, 1H), 2.95～2.74 (m, 2H), 1.29 (t, J = 7.1Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, TMS, ppm): δ 171.17, 169.09, 142.36, 139.77, 137.11, 134.82, 129.61, 128.55, 128.18, 127.47, 127.38, 127.19, 126.29, 122.23, 119.38, 118.58, 112.22, 108.93, 62.12, 53.42, 44.66, 21.63, 14.63. ESI-MS: Calcd. for $C_{27}H_{24}N_2O_3$ $[M+Na]^+$ 447.1787. Found: 447.1748.



Reagents and conditions: i OHCCOOH, pH 3～4, H_2O/KOH, 8 h;

ii 1,4-dioxane/NaOH (2 mol/L) (1:1), 20 h; iii $SOCl_2$. EtOH, reflux

Scheme 2. Synthesis of the target compound 5

2. 3 Structure determination

The clear white crystal of the compound was obtained by recrystallization from methanol-ethyl acetate (v/v = 1:6). The crystal with dimensions of 0.23mm × 0.20mm × 0.15mm was chosen for X-ray diffraction analysis.

The data were collected on a Super Nova, Dual, Cu at zero, Eos diffractometer equipped with a graphite-monochromatic Mo*Ka* radiation (λ = 0.7107 Å) at 294.64(10) K. The structure was solved by direct methods with ShelXS[14] and refined with the ShelXL[15] refinement package using Least-squares minimization. All hydrogen atoms were positioned geometrically and refined using a riding model, with U_{iso}(H) = nU_{eq}(carrier atom), where n = 1.5 for the methyl hydrogen groups and 1.2 for the C(H), C(H, H) and all N(H) groups. The final R (reflections) = 0.0551, wR = 0.1061 ($w = 1/[\sigma^2(F_o^2) + (0.0492P)^2 + 1.21P]$, where $P = (F_o^2 + 2F_c^2)/3$), S = 1.036, $(\Delta/\sigma)_{max}$ = 0.000, $(\Delta/\rho)_{max}$ = 0.12 and $(\Delta/\rho)_{min}$ = –0.18 e/Å^3.

2. 4 Pharmacological tests

The bioactivities of the title compound were tested by MTT assay. HeLa, A549, Hep-G2 and MCF-7 were selected for the experiment[16]. All cell lines were kept at 37 ℃ in 5% CO_2 in RPMI-1640 medium, and supplemented with 10% fetal calf serum. The solution of the compound with different concentrations was added into 96-well plates after 24 h with fascaplysin as the positive group. After 48 h, 10 μL of MTT solution (5 mg/mL in PBS) was added into each well and incubated for 4 h. Then, the medium was removed and 150 μL DMSO was added to dissolve the blue-colored formazan in 10 min. The absorbance was detected at 570 nm to calculate the inhibition rate (%) and the IC_{50} was defined.

3 RESULTS AND DISCUSSION

The structure of the title compound **5** was confirmed by ^{1}H NMR, ^{13}C NMR, EI-MS and elemental analysis. ^{1}H NMR exhibited a characteristic single at δ 11.05 ppm which was assigned to the proton of NH group. ^{13}C NMR also displayed distinctive peaks at 171.17 and 169.09 ppm, corresponding to the carbonyl carbon. And the ESI-mass spectrum showed the molecular ion peak as the base peak at m/z 447.1748[M+Na]$^+$. Its structure was further determined by single-crystal X-ray diffraction analysis. The single-crystal X-ray crystallographic analysis reveals that compound **5** crystallizes in orthorhombic space group *Pbca*. The selected bond lengths, bond angles and torsional angles are shown in Tables 1 and 2. And the hydrogen bonds are given in Table 3. The molecular structure of the title compound with atomic numbering scheme is shown in Fig. 1, and Fig. 2 depicts the molecular packing and hydrogen bonds in a unit cell.

As shown in Table 1, for the title compound **5**, the bond lengths of C(2)–N(1) (1.377(3) Å), C(3)–N(1) (1.372(4) Å) and C(12)–N(2) (1.345(3) Å) are shorter than the typical C–N (1.47 Å) and longer than the typical C=N (1.35 Å), which confirm these bonds have some characters of a double or conjugated bond. The

bond angles of N(2)–C(12)–C(13), O(1)–C(12)–N(2) and O(1)–C(12)–C(13) are 117.4°, 121.7° and 120.9°, with the sum to be nearly 360°, which indicates sp^2 hybridization of the C(12) atom. Respectively, the crystal structure is not coplanar according to the torsion angles C(2)–C(1)–C(25)–O(2) of –115.2° and N(2)–C(1)–C(25)–O(2) of 7.2°. Moreover, the dihedral angle between benzene rings C(13)～C(18) and C(19)～C(24) is 27.25°(96). What's more, the indole ring plane (N(1)～C(2)) makes dihedral angles of 46.34°(78) and 19.10 °(83) with benzene rings C(13)～C(18) and C(19)～C(24), which means that these two benzene planes and the indole ring plane are not coplanar. Furthermore, the intermolecular hydrogen-bonding interaction N(1)–H(1) ··O(1) in the crystal links two molecules together. Moreover, they are further linked to form an extensive network by C(11)–H(11B)···π (H(11B)–Cg(5) = 2.95 Å), C(17)–H(17)···π (H(17)–Cg(1) = 2.86 Å), C(17)–H(17)···π (H(17)–Cg(3) = 2.95 Å) and weak π-π stacking. Among them, the hydrogen bonds between nitrogen atoms as donors and oxygen atoms as acceptors, N(1)–H(1)···O(1), with the N(1) to O(1) distance of 2.907 Å, help to stabilize the structure to the extreme.

4 BIOLOGICAL ACTIVTIES

Four human tumor cells (A549, HeLa, HepG2 and MCF-7) were used for cytotoxicity test, and the *in vitro* antitumor activities of the title compound were evaluated by the MTT assay. Fascaplysin was used as a positive control. As described in Table 4, it displays different degree of inhibition against A549, HeLa, HepG2 and MCF-7 with the IC_{50} values of 10.45 ± 1.5, 4.71 ± 0.88, 10.85 ± 1.49 and 10.19 ± 1.80 μmol/L. However, compound **5** exhibits better inhibitory activity against Hela. Hence, the target derivative is expected to be developed as a novel antitumor agent, and further structural optimization is undergoing.

Table 1. Selected Bond Lengths (Å) and Bond Angles (°) for the Target Compound

Bond	Dist.	Bond	Dist.
O(1)–C(12)	1.231(3)	C(1)–C(25)	1.519(3)
O(2)–C(25)	1.189(3)	C(2)–N(1)	1.377(3)
O(3)–C(25)	1.327(3)	C(3)–N(1)	1.372(4)
O(3)–C(26)	1.457(3)	C(7)–C(8)	1.404(3)
N(1)–C(2)	1.377(3)	C(8)–C(9)	1.424(3)
N(1)–C(3)	1.372(3)	C(9)–C(10)	1.496(3)
N(2)–C(1)	1.455(3)	C(10)–C(11)	1.510(4)
N(2)–C(11)	1.480(3)	C(12)–C(13)	1.495(3)
N(2)–C(12)	1.345(3)	C(12)–N(2)	1.345(3)
C(1)–C(2)	1.498(3)		

Angle	(°)	Angle	(°)
C(25)–O(3)–C(26)	117.1(2)	C(9)–C(10)–C(11)	108.9(2)
C(3)–N(1)–C(2)	108.1(2)	O(1)–C(12)–N(2)	121.7(2)
C(1)–N(2)–C(11)	115.67(19)	O(1)–C(12)–C(13)	120.9(2)
C(12)–N(2)–C(1)	117.88(19)	N(2)–C(12)–C(13)	117.4(2)
C(12)–N(11)–C(11)	125.4(2)	C(14)–C(13)–C(12)	120.0(2)
N(2)–C(1)–C(2)	108.53(18)	C(14)–C(13)–C(18)	118.5(2)
N(2)–C(1)–C(25)	111.0719)	C(18)–C(13)–C(12)	121.4(2)
C(2)–C(1)–C(25)	113.15(19)	C(15)–C(16)–C(19)	122.0(2)
N(1)–C(2)–C(1)	123.9（2）	C(17)–C(16)–C(19)	120.9(2)
C(9)–C(2)–N(1)	110.1(2)	C(20)–C(19)–C(16)	122.0(3)
C(9)–C(2)–C(1)	125.8(2)	C(24)–C(19)–C(16)	120.6(3)
N(1)–C(3)–C(4)	129.4(3)	C(19)–C(20)–C(21)	120.6(3)
N(1)–C(3)–C(8)	108.1(2)	O(2)–C(25)–C(1)	125.0(2)
C(2)–C(9)–C(10)	121.6(2)	O(3)–C(25)–C(1)	110.5(2)
C(8)–C(9)–C(10)	131.5(2)	O(2)–C(26)–C27)	108.1(3)

Table 2. Selected Torsional Angles (°) for the Target Compound

Angle	(°)	Angle	(°)
N(1)–C(2)–C(9)–C(10)	–177.4(2)	C(12)–N(2)–C(11)–C(10)	–127.4(3)
N(2)–C(1)–C(2)–N(1)	167.1(3)	C(12)–C(13)–C(14)–C(15)	–176.4(3)
N(2)–C(1)–C(2)–C(9)	–8.1(3)	C(12)–C(13)–C(18)–C(17)	177.5 (2)
N(2)–C(1)–C(25)–O(2)	7.2(4)	C(15)–C(16)–C(17)–C(18)	4.2(4)
N(2)–C(1)–C(2)–O(3)	–171.92(19)	C(15)–C(16)–C(19)–C(20)	27.0(4)
C(1)–N(2)–C(11)–C(10)	–64.5(3)	C(15)–C(16)–C(19)–C(24)	–151.4(3)
C(1)–N(2)–C(12)–O(1)	1.6 (4)	C(16)–C(17)–C(18)–C(13)	–1.3(4)
C(1)–N(2)–C(12)–C(13)	–179.5(2)	C(16)–C(19)–C(20)–C(21)	–178.5(3)
C(2)–C(1)–C(25)–O(2)	–115.2(3)	C(16)–C(19)–C(24)–C(23)	–178.2(2)
C(11)–N(2)–C(1)–C(25)	–84.4(3)	C(17)–C(16)–C(19)–C(20)	–155.2(3)
C(11)–N(2)–C(12)–O(1)	169.4(2)	C(17)–C(16)–C(19)–C(24)	26.5(4)
C(11)–N(2)–C(12)–C(13)	–11.6(4)	C(25)–C(1)–C(2)–N(1)	–69.1(3)
C(12)–N(2)–C(1)–C(2)	–150.4(3)	C(25)–O(3)–C(26)–C(27)	–179.8(3)
C(12)–N(2)–C(1)–C(25)	84.6(3)	C(26)–O(3)–C(25)–C(1)	180.2(2)

Table 3. Hydrogen Bond Lengths (Å) and Bond Angles (°)

D–H ··A	d(D–H)	d(H ··A)	d(D ··A)	∠DHA
N(1)–H(1) ··O(1)#1	0.860	2.101	2.907	155.83
C(11)–H(11B) ··Cg(5)#2	0.970	2.950	3.777(3)	143
C(17)–H(17) ··Cg(1)#3	0.930	2.860	3.459(3)	123
C(17)–H(17) ··Cg(3)#4	0.930	2.950	3.756(3)	146

Symmetry codes: #1: *–x*, *–y*+1, *–z*+1; #2: *x*, 1+*y*, *z*, #3: *x*, *–*1+*y*, *z*; #4: *x*, *–*1+*y*, *z*.

Cg(1): N(1), C(3), C(8), C(9), C(2); Cg(3): C(3)～C(8); Cg(5): C(19)～C(24)

Table 4. Inhibition of the Cell Growth (μM)

Compound	Cytotoxicity (IC50, μM)			
	A549	HeLa	HepG-2	MCF
Compound **5**	10.45 ±1.5	4.71 ±0.88	10.85 ±1.49	10.19 ±1.80
Fascaplysin	1.05 ±0.08	0.24 ±0.05	0.75 ±0.04	0.93 ±0.08

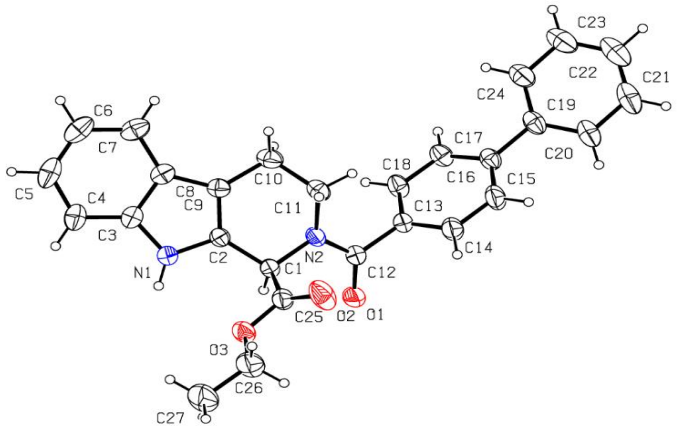


Fig. 1. Structure of the title compound

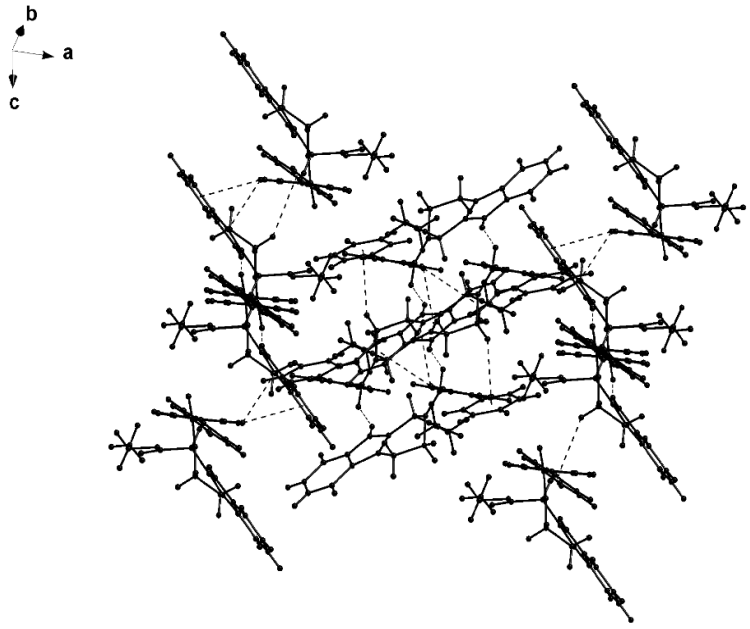


Fig. 2. Three-dimensional structure of the title compound showing hydrogen bonds

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The title compound ethyl was designed and synthesized, and its structure was further confirmed by single-crystal X-ray diffraction. The crystal structure was mainly stabilized by two kinds of hydrogen bonds and the bioactivity was analyzed by MTT assay. It has good antitumor activity against Hela *in vitro*.